APAC 3/5/01
SS #3

Cockroach allergen standardization - preliminary report

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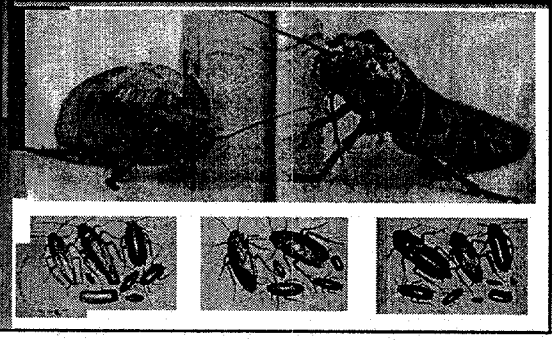
e standardization of new allergens: pact criteria for candidate allergens

- Availability of stable, preferably lyophilized material for use as long-term reference extracts.
- Consistency of currently marketed product.
- Widespread use as a diagnostic and/or therapeutic reagent in the U.S.
- Number of manufacturers producing the product.
- Potential use in immunotherapy (higher score) or diagnostics (lower score).
- Public health impact of correct diagnosis and/or adequate treatment.

ckroaches



y is cockroach allergy important?

- Ubiquitous
- Difficult to control
- Associated with asthma
- Several cloned allergens

ase I - Laboratory

- Develop/adapt methods for allergen determination
- Compare allergen content of different lots
- If commercial products are highly consistent, re-consider impact
- If commercial products are comparable to "best" material, re-consider impact

eliminary Studies

- Obtain multiple lots for study from manufacturers
- Characterize and compare extracts
- Identify target allergens

3735 S1 07 - FDA (Jay Slater)

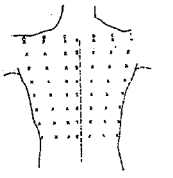
ɔals

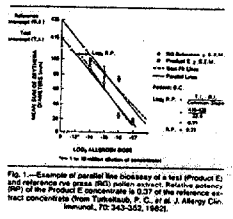
- Determine consistency of available US products:
  - protein content
  - specific allergen content
  - overall allergenicity
- Determine best lot release measures

tracts used as reference

- E2-Cg and E2-Ca
- Previously characterized
- Limited skin test data
- Lyophilized; available in large quantities

D_{50}EAL method





$_{50}$EAL method

D50 = 14 ➡ 100,000 BAU/mL

- BAU/mL = 100,000 x 3 $^{(D50 - 14)}$

ɔckroach clinical data

German	E2-Cg			
Σ E	n	D50	BAU/mL	95% CI
0	11	11.2	4429	998-19644
50	7	11.9	9423	1077-82482

American	E2-Ca			
Σ E	n	D50	BAU/mL	95% CI
0	10	10.3	1681	342-8260
50	7	10.3	1733	201-14973

ɔckroach extracts studied

- From all nine allergen extract manufacturers

	aqueous	50% gly
American	4	11
German	2	9

ethods Used:

- Protein Assays
  - BCA
  - Bradford
  - ninhydrin
- SDS-PAGE
- ELISA
- Western Blot

otein concentrations (ninhydrin thod)

Glycerinated	American	Mean	9.29
		SD	7.05
		Range	3.67 - 22.88
	German	Mean	4.44
		SD	2.61
		Range	2.44 - 10.42
	Aqueous	Mean	6.80
		SD	9.73
		Range	0.09 - 24.82

otein concentrations (ninhydrin thod) of candidate reference extracts

- E2-Cg 25 mg/mL
- E2-Ca 30 mg/mL
- Therefore, we scaled up the allergen levels and relative potencies to the reference protein concentration

Correlation of protein assays

	ninhydrin vs. BCA	ninhydrin vs. Bradford	BCA vs. Bradford
aqueous	0.99	0.95	0.92
glycerin	0.73	0.37	0.35

rrelation of Protein Assays

- Glycerin interferes with the Bradford assay and, to a lesser degree, the BCA assay (Richman PG, Cissel DS, J Biol Stand 1988; 16:225-38)
- Ninhydrin protein concentrations were used for remainder of analyses

otein assays - conclusions

Protein concentrations of the commercial extracts vary widely, and are lower than the protein concentrations of the references

- Glycerin interferes with the Bradford and, to a lesser degree, the BCA assay

cation of Relevant Allergens

German cockroach

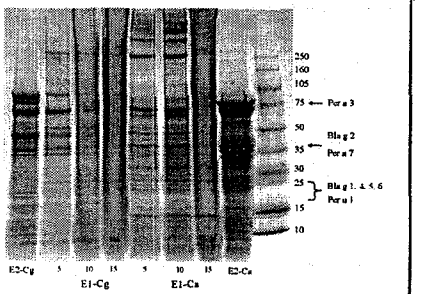
• Bla g 1	20-25 kD	Bd90k
• Bla g 2	36 kD	aspartic protease
• Bla g 4	21 kD	calycin
• Bla g 5	25 kD	glutathione transferase
• Bla g 6	27 kD	troponin C

- American cockroach

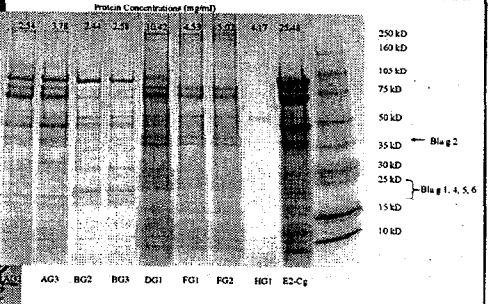
• Per a 1	20-25 kD	Cr-PII
• Per a 3	72-78 kD	Cr-PI
Per a 7	37 kD	tropomyosin

CBER Cockroach References

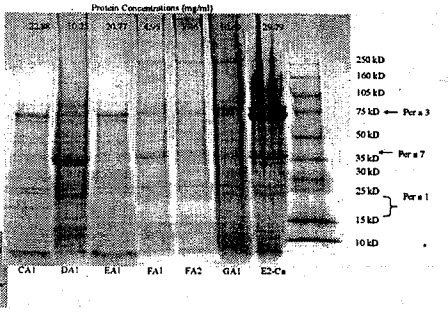
E2-Cg , E2-Ca, E1-Cg and E1-Ca



ycerinated German Cockroach



lycerinated American Cockroach



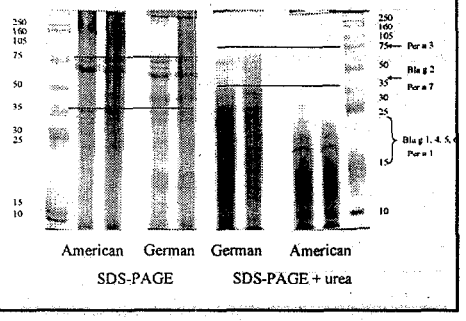
DS vs. urea

SDS is a detergent - denatures proteins and confers uniform charge density

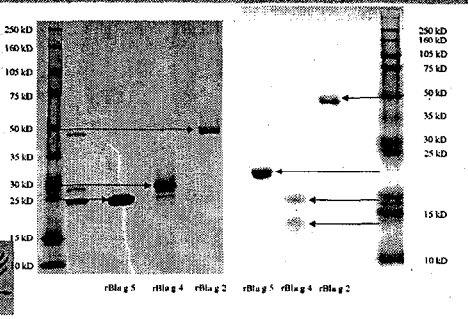
4+ M urea is a stronger denaturant

- used in addition to SDS in gels

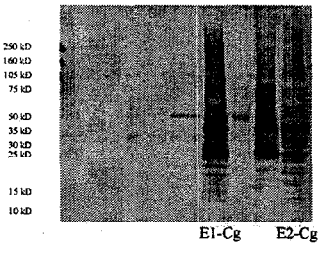
ost of the bands above 35 kD appear in 4M urea



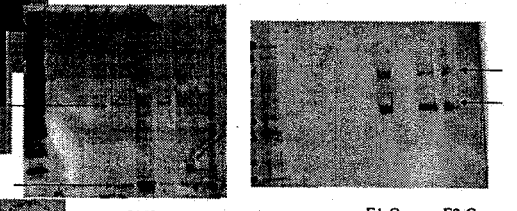
combinant Allergens



mmunoblots - pooled allergic ra



mmunoblots - monoclonal tisera



elative Potency of All Cockroach Extracts

			RP	RP (scaled)
Glycerinated	American	Mean	0.07	0.05
		SD	0.05	0.04
		Range	0 to 15	0 to 12
	German	Mean	0.05	0.22
		SD	0.06	0.19
		Range	0 to 18	01 to 46
	Aqueous	Mean	0.00	0.03
		SD	0.00	0.05
		Range		

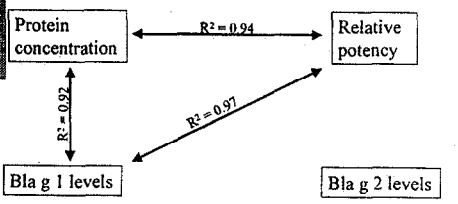
ecific antigen assays

- *Bla g 1 and Bla g 2 levels measured by ELISA using monoclonal antibodies*
- *Results normalized for protein content*

Bla g 1 and Bla g 2 Levels in glycerinated German cockroach

	Bla g 1*, U/ml	Bla g 2*, U/ml
Mean	3503	43774
SD	1094	21815
Range	2218 to 4854	8237 to 66439
E2-Cg	13829	8306

rrelation of protein ncentrations and ELISA results



nclusions

- *Commercially available cockroach allergen extracts:*
  - *vary widely in protein content, Bla g 2 content, SDS-PAGE banding pattern, and overall allergenicity.*
  - *appear to be less potent and contain less Bla g 1 than the candidate reference extracts*

ture directions

- *IDE$_{50}$AL skin testing to identify the best* in vitro *potency measure*
- *Selection of a reference standard*

knowledgements

- *Melissa L. Patterson*
- *Jonny Finlay*